

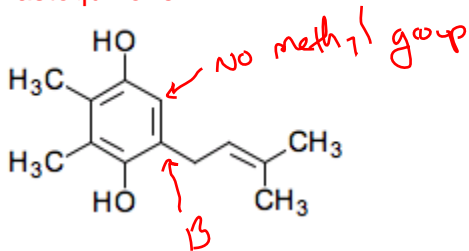
Problem Set 7

(Due March 11th)

1. Using the KEGG Pathways website, navigate to the Photosynthesis page. You should see a cartoon image of all the relevant proteins and complexes.

a. What is the name and structure of the quinone that carries electrons from PSII to Cyt b_6f ?

Plastoquinone



How is this different than the quinone that is important in mitochondrial electron transport? It lacks a methyl group at the indicated position and the carbon chain at position B is much shorter

b. For every NADPH that is produced in the stroma, how many protons are produced in the lumen? 6. 2 from H_2O and 4 from b_6f complex pumping across the membrane

c. How many protons are required to synthesize 1 ATP? 3

d. Click on the Antenna Proteins link: How many light harvesting proteins are associated with each photosystem? What are their names?

PSI \rightarrow 5 \rightarrow Lhca1, Lhca2, Lhca3, Lhca4, Lhca5

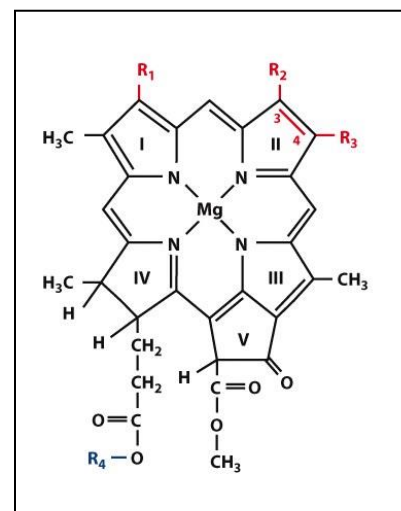
PSII \rightarrow 7 \rightarrow Lhcb1, Lhcb2, Lhcb3, Lhcb4, Lhcb5, Lhcb6, Lhcb7

2. Describe the four fates of excited electrons and indicate which are important for photosynthesis. Justify your answer.

- Internal Conversion – excited electrons relax back to the ground state (or a lower excited state) and release energy as heat in the process.
- Fluorescence – an absorbed photon relaxes to its excited state by emitting a photon (typically of lower wavelength because is a much slower process than internal conversion)
- Exciton Transfer – excited electron is transferred to a nearby molecule. This process leaves a hole on the original molecule.
- Photooxidation – a donor molecule is oxidized and generates an electron to a nearby molecule (this is typically used to fill a hole after exciton transfer).

3. What is the difference between Chlorophyll A, Chlorophyll B, and pheophytin?

Chlorophyll a, b and pheophytin are all tetrapyrroles with the general structure shown below. The only difference between chlorophyll a and b is the functional group at the 3 position (methyl and formyl, respectively). Pheophytin is a chlorophyll molecule that lacks the Mg^{2+} (replaced with 2 protons)



4. As we discussed in class, there are roughly 300 chlorophyll molecules for every photosynthetic reaction center. Noting that not all of these chromophores are part of the reaction center, what is the role of these additional molecules? Please discuss the proteins that harbor these additional chlorophyll molecules and how your answer to problem 1 is relevant.

There are numerous light harvesting complexes that surround photosynthetic reaction centers. This increases the number of photons that can be absorbed. These complexes are largely decorated with chlorophyll molecules and a number of other chromophores (e.g. lutein and β carotene). The absorbed photons at these light harvesting complexes are transferred to a photosynthetic reaction center by a series of exciton transfer processes.

5. The Z scheme and the red drop are two important features of photosynthesis. Describe what is meant by each of these terms. In addition, please describe an experiment that uses the red drop to confirm the Z scheme. (include a sketch of the data and explain what it means).

The Z-scheme is a term that describes the coupled nature of PSII and PSI. An excited electron from PSII gets transferred to PSI and is subsequently excited at that reaction center. The red drop is a term that describes the sharp drop off in photosynthesis efficiency when red light is the primary energy source. This is due to the fact that red light does not excite PSII very well.

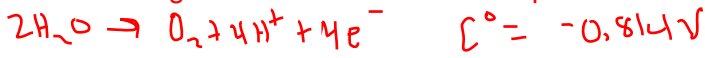
The experiment that confirms the Z scheme was described in class. It relies on the signal that is generated when cyt f is oxidized. Using red light as the sole light source results in an increased signal as cyt f transfers its electrons to PSI. This results in an oxidation and increased signal. However, when the red light is supplemented with yellow-green light, the signal decreases, indicating that cyt f is being reduced. The only possible explanation is that PSII is providing electrons to cyt f.



6. Energy and efficiency:

a. How many photons of red light ($\lambda = 650 \text{ nm}$) are needed to produce 1 O_2 from 2 H_2O ?

Noting that water oxidation is coupled to NADP+ reduction:



overall reaction: $\epsilon^\circ = -1.134 \text{ V}$

$$\Delta G = -nF\epsilon^\circ = -4(96485)(-1.134)$$

$$\Delta G = 437.66 \text{ kJ}$$

A single photon of 650 nm light:

$$\frac{E=hc}{\lambda} = \frac{6.626 \times 10^{-34} (3 \times 10^8)}{650 \times 10^{-9}} = 3.058 \times 10^{-19} \frac{\text{J}}{\text{photon}}$$

Converting to molar energy:

$$3.058 \times 10^{-19} \frac{\text{J}}{\text{photon}} \times \frac{6.022 \times 10^{23} \text{ photons}}{\text{mol}} = 184.2 \text{ kJ/mol}$$

The number of photons is the ratio:

$$437.66 \frac{\text{kJ}}{\text{mol}} \times \left(\frac{\text{mol photons}}{184.2 \text{ kJ}} \right) = 2.4 \text{ photons}$$

b. Noting that it takes 8 photons to carry out this process, how efficient is photosynthesis?

$$\frac{2.4}{8} \times 100 = 30\% \text{ efficient} \quad 70\% \text{ of energy is lost!}$$

c. So clearly energy is lost in this process. Propose a reason for the energy loss.

Exciton transfer and internal conversion are both huge pieces of photosynthesis. Both of these processes provide avenues for energy loss – internal conversion results in loss of energy in the form of heat and exciton transfer can release a photon from the antenna complex prior to transfer to a reaction center

d. For every O_2 that is produced at the OEC, 12 protons are accumulated in the thylakoid lumen. Account for all of these protons (problem 1 should help with this) 2 H_2O are required for 1 O_2 , so 4 H^+ . For every O_2 , 4 H^+ are pumped by the b_6f complex, so another 8.
 $8+4=12$

e. Calculate how many ATP are produced per photon of red light ($\lambda = 650 \text{ nm}$) – remember to account for the efficiency that you determined in 5b.

$$\frac{1 \text{ ATP} \mid 3 \text{ H}^+ \mid \text{O}_2 \mid 2.4 \text{ photons} \mid 100}{1 \text{ ATP} \mid 12 \text{ H}^+ \mid \text{O}_2 \mid 30\%} = 2 \text{ photons/ATP}$$

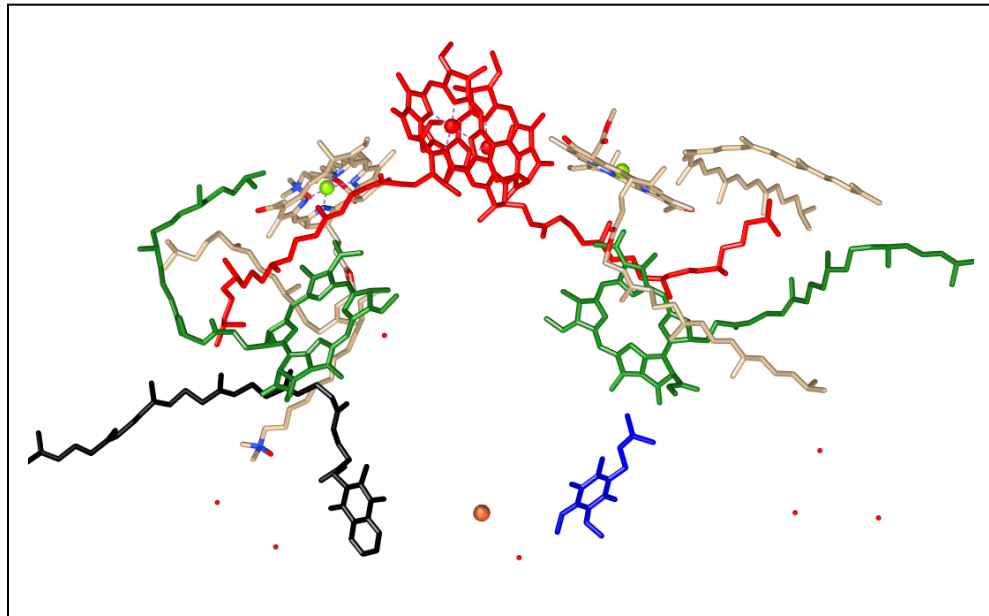
f. Noting that it takes 30.5 kJ mol^{-1} to make ATP from ADP and Pi, how many photons ($\lambda = 650 \text{ nm}$) should be theoretically needed?

$$30.5 \frac{\text{kJ}}{\text{mol}} \times \frac{1 \text{ photon}}{184.2 \frac{\text{kJ}}{\text{mol}}} = 0.166 \text{ photons to make 1 ATP}$$

Not very efficient, eh?

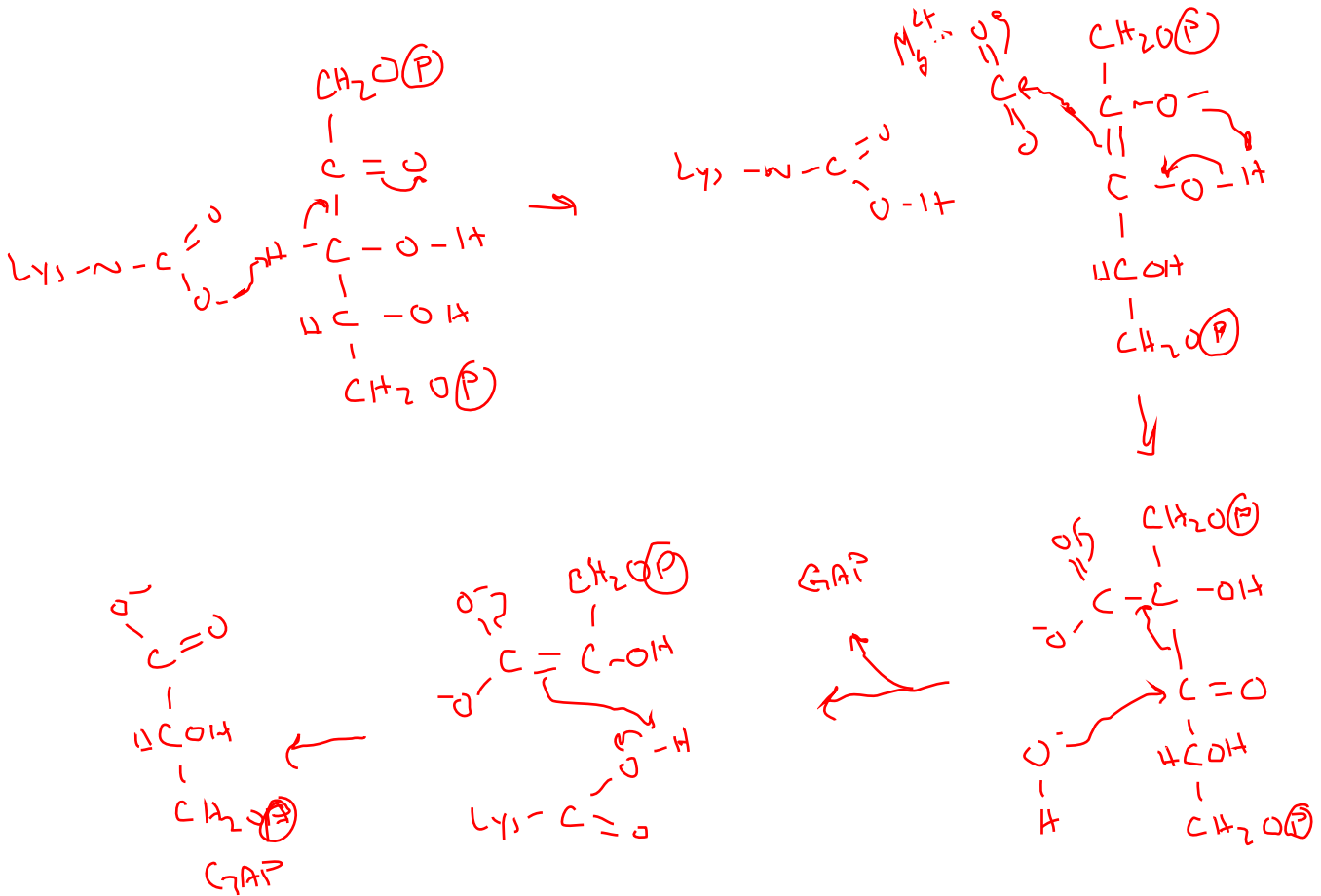
7. Photosynthetic Reaction Center:

- a. Using the crystal structure of the photosynthetic reaction center from *Rps. viridis*, (pdbID 1PRC), make an image that shows just the redox cofactors (you should be able to make it resemble Figure 29-57 in your book). Color the Special Pair red, menaquinone black, ubiquinone blue and the pheophytins green.

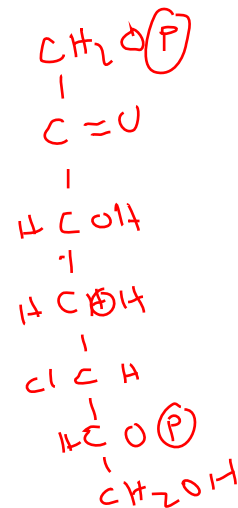
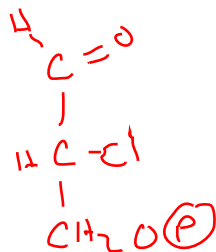
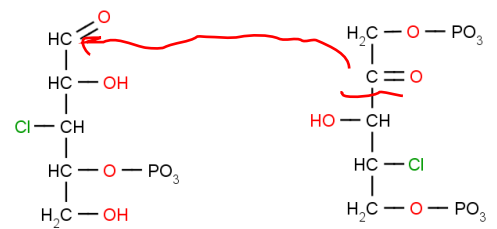


- b. What is the distance between Mg^{2+} ions in the special pair? What is the reason that these two chlorophyll molecules are so close? **7.58 angstroms. These two chlorophylls are so close so that the redox potential is modulated in a way that electrons from the light harvesting complexes will be transferred to this special pair in a thermodynamically favorable way (ϵ^0 special pair > harvesting complex).**
- c. Please show the sequence of electron transfer steps in this reaction center and list the approximate times it takes for each step.
Special pair \rightarrow **pheophytin** (~3 ps)
Pheophytin \rightarrow menaquinone (Q_A) (200 ps)
menaquinone (Q_A) \rightarrow **ubiquinone** (Q_B) (100 μ s)
- d. Recalling that fluorescence and internal conversion are very slow processes (~ 200 μ s), what is the significance of the rate of electron transfer in the scheme you determined in 7c? **In half the time it takes for the electron to fluoresce and relax back to the ground state, the excited electron is transferred through all cofactors and stabilized on ubiquinone for shuttle to cytochrome bc_1 (b_6f in plants). If the electron were allowed to be released via fluorescence, the electron transport chain would terminate and photosynthesis would fail.**
- e. What is the source of electrons that fill the hole that is formed upon photon absorption in PSI and PSII? **In PSI, the electron hole is filled by a reduced plastocyanin (PC), which is a result of electron transfer FROM PSII through Cyt. b_6f . The electron hole on PSII is filled by oxidation of water to O_2 .**
- f. What is the fate of electron that is excited at PSII? **$NADP^+ \rightarrow NADPH$**

8. Draw a reaction mechanism for the carbon fixation step of the Calvin Cycle.

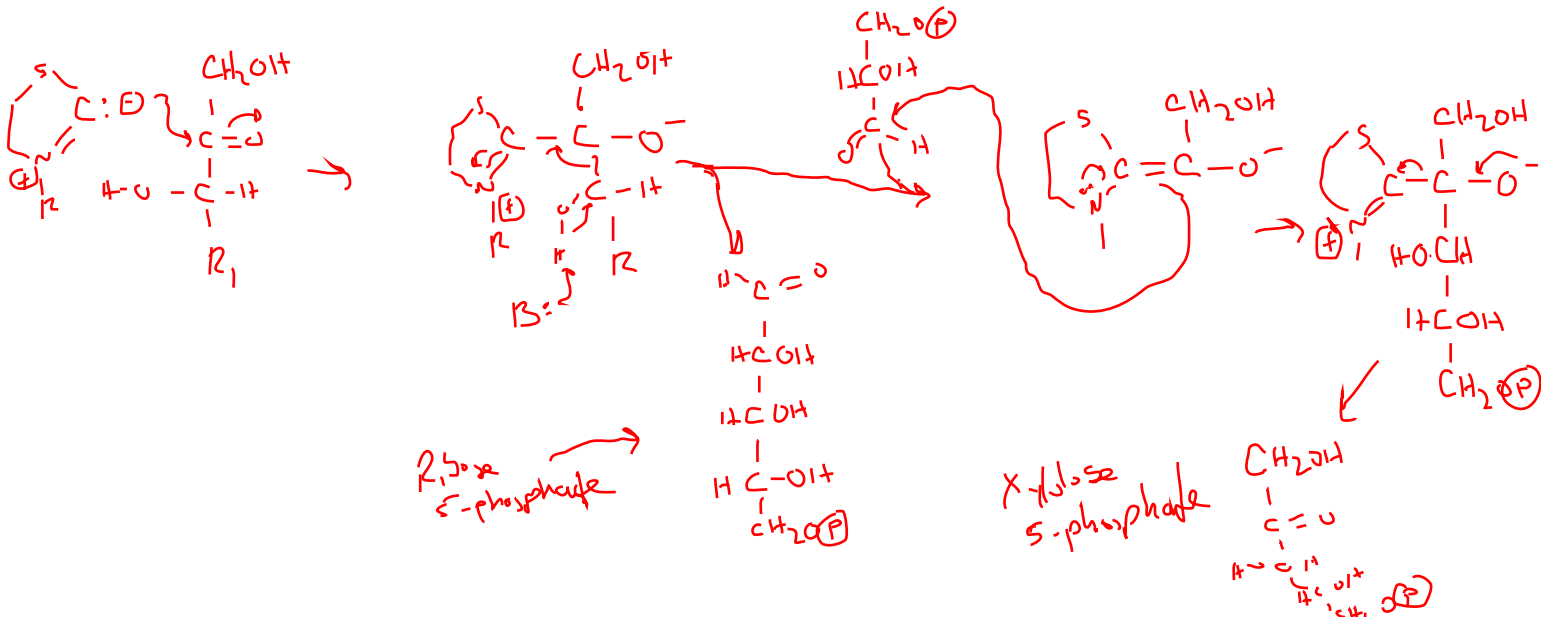


9. Predict the product of a transketolase reaction between the two molecules to the right.



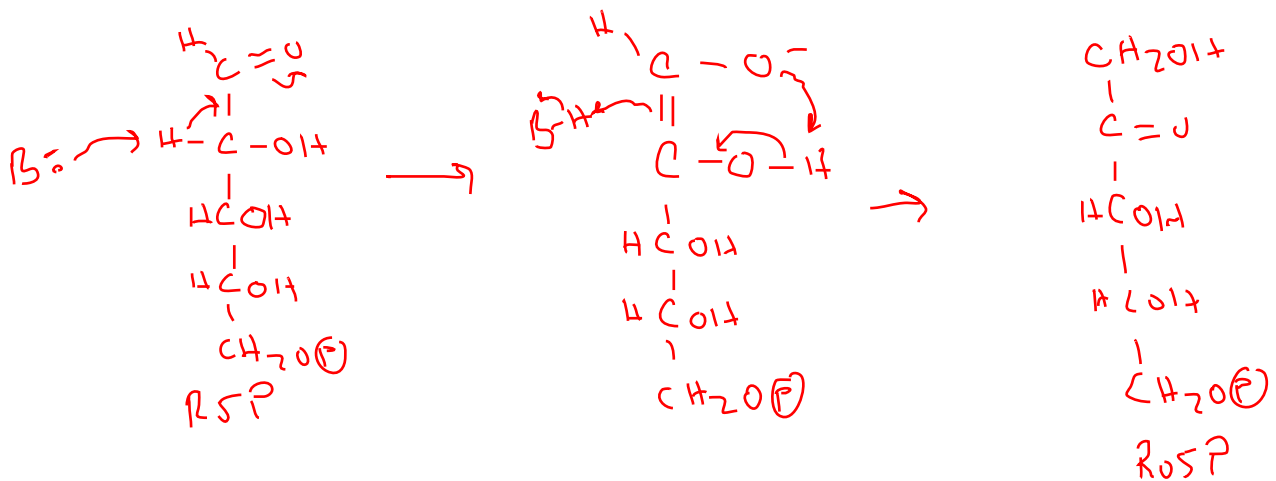
10. As noted in class, the Calvin Cycle image shown in the lecture slides contains an error in the 2nd transketolase reaction (S7P + GAP).

a. Show the mechanism of this reaction and determine what the products should be.



b. The aldehyde that is produced in this reaction can be converted directly to Ru5P in a mechanism that is identical to one of the steps in glycolysis. Identify the enzyme that catalyzes this transformation and predict a mechanism.

R5P isomerase



11. Triose phosphates are the direct product of the Calvin Cycle

a. Which triose phosphates are produced? **GAP or DHAP**

b. How can these compounds be converted to hexose phosphates and pentose phosphates?

GAP+DHAP → FBP (aldolase)

FBP → F6P (FBPase-1)

F6P → G6P (PGI)

G6P → pentoses (you can use your book or KEGG to see that this happens)

12. Read the manuscript attached to answer the following questions:

- a. Ferredoxin is important in a wide variety of cellular processes including carbon assimilation, nitrogen assimilation, sulfur assimilation and cellular redox control. It participates in these processes through interactions with a variety of proteins. For each of the pathways listed above, identify one or more partner proteins. Which of these interactions is important in photosynthesis?
Carbon assimilation → Ferredoxin-NADP⁺ reductase (FNR) → this one is important in photosynthesis
Nitrogen assimilation → nitrite reductase and glutamate synthase
Sulfur assimilation → Sulfite reductase (SiR)
Redox regulation → ferredoxin-thioredoxin reductase
- b. Describe the goals of this study. It has been postulated that Fd interacts with partner proteins based on surface charge. The authors want to determine what residues dictate specificity for FNR vs. SiR
- c. What amino acids were mutated in this study and why? The authors selected acidic residues that are predicted to decorate the exterior of the protein. D27, E30, D58, D61, D66, D67, E71, E72, D85, E93. All were mutated to the corresponding neutral residue (E→Q and D→N) or basic amino acids in one case.
- d. How did the investigators confirm that site-directed mutants don't significantly perturb protein structure? They used CD spectroscopy and determined the redox potential, which would deviate significantly if the structure was perturbed.
- e. Figure 5 confirms that Fd and SiR form a complex at pH 7.5. Describe how this conclusion was reached based on the data. The proteins were added together in a gel filtration chromatography experiment. If the proteins don't interact, two independent peaks should show up corresponding to Fd and SiR. However, the authors saw a peak that corresponded to a complex bigger than either of the individual proteins; the only reasonable explanation is that a complex is formed.
- f. Compare the results from Figure 6 to the activity data from Table 2. Describe any correlation observed between these two pieces of experimental data. It's expected that the mutants with decreased affinity for each other should have dampened activity (since the activity is a measure of electron transfer between the two proteins, which requires an interaction). Indeed, the mutants that have a dampened affinity also have a decreased activity.